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CONTROL OF MICROBIOLOGICAL HAZARDS IN THE LABORATORY

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CONTROL OF MICROBIOLOGICAL HAZARDS IN THE LABORATORY

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ABSTRACT

Occupational disease occurs at a significant frequency among laboratory workers handling infectious cultures and infected animals. The severity of laboratory infections, in terms of case fatality rate, is higher than the fatality rates for motor vehicle accidents. The handling of "normal" animals also presents a risk of zoonose infection. Although the precise causes of most laboratory infections are not recognized in terms of specific accidents and events, infection by the respiratory route following unconscious release of microbial aerosols is typical. Because these aerosols originate at the laboratory working surface, containment devices such as ventilated cabinets are of prime importance in preventing infections. An adequate safety program includes not only the necessary safety equipment and proper building facilities, but also the use of correct techniques and vaccination of laboratory personnel. The laboratory administration should provide the program with safety regulations, a means of reporting and analyzing accidents, and procedures for selecting and training qualified employees. In the campus situation there is a dual responsibility for microbiological safety: (a) that of preventing occupational-infections among instructors, students and scientists and (b) that of training the student for his future safety in microbiological operations. Adequate safety education will serve both of these needs.

I. INTRODUCTION

Almost since microbiology began as a science, accidental infections resulting from laboratory manipulation of pathogenic microorganisms were recognized and recorded. Louis Pasteur finally disproved the theory of spontaneous generation in 1861, and in the 1870's began his studies with disease-producing organisms. Robert Koch solved the problem of growing pure bacterial cultures in the laboratory in 1881, and in the following two years discovered the etiologic agents of tuberculosis and cholera. The organisms producing typhoid fever were identified in 1880, and five years later, in 1885, two cases of occupationally acquired typhoid fever were recorded in the German Imperial Health Service. In 1893 another case of laboratory-acquired typhoid fever was recorded in Germany and a case of tetanus was reported in France. In 1903 the first recorded case of blastomycosis following an accidental self-inoculation occurred. Today, more than 70 years later, the problem of accidentally acquired laboratory disease is still with us. It is not at all unusual to see reports of laboratory infections in the current medical literature. Moreover, through the years the frequency of reports of laboratory infections appears to have increased as the science of microbiology has expanded.

Microbiological safety, in its simplest form relates to the precise control of the microbial elements in any particular environment; that is, microbiological environmental control. Its application in laboratories where pathogenic cultures or infected animals are being used will help to prevent infections in laboratory workers.

A second reason for microbiological environmental control relates to protecting the validity of the experiment. In the absence of suitable controls, laboratory results can be confounded by accidental or unintentional transfer of infectious microorganisms from animal to animal or from test tube to test tube.

A third reason for microbiological environmental control is to protect man from infection by laboratory animals not known to be infected. Human infections may result from any laboratory use of animals. For example, laboratory animals used by a psychology department for behavioral studies can present a human infectious hazard if they carry an unrecognized disease and if microbiological environmental control is inadequate. Tuberculosis in monkeys is a good example of this.

This paper is directed primarily toward controlling microbiological hazards in the infectious disease laboratory to prevent occupational infections. We should, however, realize that other applicable areas exist.

We shall begin by considering what is known about the frequency and severity of laboratory infections and what we might learn from these data that

will help in assessing our own laboratory programs. Next, we will review the known and probable causes of laboratory infections. Then we will consider the available methods for preventing laboratory infections, dealing with five approaches to laboratory safety and giving some specific recommendations for each. Finally, we will discuss the educational needs and the future prospects for microbiological laboratory safety in the campus situation.

II. INCIDENCE AND SEVERITY OF LABORATORY INFECTIONS

German physicians were the first to publish collected cases of laboratory illnesses. In 1915 Kisskalt[1] summarized information from 50 cases of laboratory-acquired typhoid fever that had occurred in Germany since 1895. In 1929 he published a summary of 59 cases of typhoid fever and 24 other laboratory-acquired diseases occurring between the years 1915 and 1928.[2] In the late 1930's, Draese[3] published the results of an investigation of 111 laboratory infections, with 9 fatalities, occurring in Germany between 1930 and 1937. However, Draese, in his survey, declined to list laboratory infections of Weil's disease (leptospirosis) and yellow fever because of their high frequency.

Through the years, several hundred publications have mentioned approximately 6000 laboratory infections. The largest single collection of cases was that published in 1951 by Sulkin and Pike.[4] This survey listed 1342 laboratory infections occurring in the U. S. during a 20-year period. Included were infections caused by 69 different disease agents, resulting in 39 deaths. Through a committee of the American Public Health Association, Sulkin has continued to tabulate reported cases; the total number of cases now stands 2348 with 107 deaths.[5]

While these reports and surveys illustrate that there can be a microbiological safety problem in infectious disease laboratories, they give us little concrete information in terms of frequency or severity rates. In fact, probably only a fraction of the laboratory-acquired infections are ever reported in the literature, and it sometimes is difficult to prove whether or not a disease actually was acquired during laboratory work. An even more illusive factor is the occurrence of accidental infection in laboratory people without their showing recognizable clinical symptoms.

In spite of the difficulties of complete reporting we can establish some useful information on frequency rates. Table I shows estimated frequency rates for several laboratory institutions and for various types of laboratories. Although these rates vary from 0.10 to 50.0 infections per million man hours, where infectious disease agents are used to an appreciable extent

TABLE I. ESTIMATED FREQUENCY RATES FOR LABORATORY INFECTIONS

Laboratory	Year	Infections per Million Man Hours	Literature Cited
European laboratory	1944-1959	50.0	6
Fort Detrick	1943-1945	35.0	
Canadian T.B. laboratories	1947-1954	19.0	
Fort Detrick	1954-1962	9.1[a]	
Research laboratories	1930-1950	4.1	4
NIH	1954-1960	3.4[b]	
CDC	1959-1962	1.3	
Hospital clinical laboratories	1953	1.0	7
Public health laboratories	1930-1950	0.4	4
Clinical laboratories	1930-1950	0.1	4

a. Includes non-lost-time infections.
b. Includes diseases of suspected occupational origin.

and where good infection detection and adequate reporting exists, an expected laboratory infection frequency rate is between 1.0 and 5.0 per million man hours. However, at any one laboratory institution the number of infections per million man hours may be expected to vary more than the number of lost-time mechanical injuries per million man hours because of the changing nature of research operations and of changes in the types of disease microorganisms employed. Mechanical hazards usually are more constantly present in the laboratory environment than infectious hazards.

Unfortunately, these rates do not give the full story of what can happen. Occasionally in the past there have been laboratory epidemics of infectious disease caused by accidents or faulty procedures that spread pathogens throughout several laboratories or through entire buildings. Table II shows some examples of laboratory epidemics.

TABLE II. LABORATORY EPIDEMICS OF INFECTIOUS DISEASE

Disease	Year	Number of Persons Infected	Literature Cited
Psittacosis	1930	11	8
Brucellosis	1938	94	9
Q Fever	1940	15	10
Murine typhus	1942	6	11
Q Fever	1946	47	12
Coccidioidomycosis	1950	13	13
Histoplasmosis	1955	18	14
Venezuelan encephalitis	1959	24	15
Tularemia	1961	5	16

The 1938 epidemic is of special interest because of student infections with brucellosis, a sometimes chronic disease. The outbreak occurred in the bacteriology building of a state university and resulted in the infection of 94 individuals, 84 of whom were students.[6] There was one fatality. Forty-five of the 94 people were hospitalized; 41 students, one laboratory stockroom attendant, one plumber, one stenographer, and one salesman. Because 316 students attended classes in this building, the attack rate appeared to be about 27 per cent. In terms of frequency rate the figure would be no less than 150 infections per million man hours! This epidemic was caused by the improper use of a centrifuge that spread air-borne contamination throughout the building.

The 1955 episode with histoplasmosis is another example. Here all 17 female students in a medical technology class apparently were infected after they carried out assigned studies involving the culturing and preparation of microscope slides of the fungus Histoplasma capsulatum. One student was ill and the others were thought to have had subclinical infections. Another male student became ill after transferring cultures of the causative microorganism.

Although laboratory epidemics of this nature have not been frequent when they do occur they can be very serious.

Severity rates for nonfatal laboratory infections in terms of days lost per million man hours, average days lost per illness, or average annual work days lost per person vary widely according to the type of disease and the quality of the medical care provided. While some diseases such as psittacosis, tularemia, and Q fever usually respond favorably to prompt medical treatment, others such as tuberculosis, brucellosis, coccidioidomycosis, Russian spring-summer encephalitis, and monkey B virus are much higher in severity and may produce permanent injury or death. The permanent physical or psychological impairment that can be produced by such diseases as tuberculosis or brucellosis, or the fact that there is no adequate medical treatment for some infectious diseases is not adequately expressed in terms of severity rates.

The severity of laboratory infections as measured by death rate is shown in Table III. The estimated combined case fatality rate for laboratory infection is 4.0 per cent. For comparison, the combined death rate for all U. S. disabling injuries in 1963 was 1.0 per cent. The class of accidents resulting in the highest death rate was motor vehicle accidents with a rate of 2.7 per cent. On this basis we can say that accidental laboratory infections have an abnormally high severity.

TABLE III. CASE FATALITY RATES FOR LABORATORY INFECTIONS

Location	Years	Infections	Deaths	Fatality Rate, per cent
Europe	1900-1957	442	33	7.47
Europe & U. S.	1900-1957	1156	57	4.93
U. S.	1930-1960	2348	107	4.56
Europe & U. S.	1944-1959	426	17	4.00
U. S.	1930-1950	1342	39	3.00
U. S. hospitals	1953	504	8	1.60
Fort Detrick	1944-1962	385	2	0.52

In addition to the previous comments on frequency and severity of laboratory infection, special hazards problems sometimes arise. Not the least of

these is the handling of monkeys and other primates. For example, since 1934 there have been 18 human cases of B virus infection identified with the handling of monkeys or their tissues.[17] Most of these cases were fatal. The causative virus apparently occurs naturally in monkeys without producing ill effects. Another example is the observation of an abnormal incidence of hepatitis among persons handling apparently healthy sub-human primates, chiefly chimpanzees. From 1953 to 1962 some 69 human cases have been documented in which primate to human transfer was suspected.[18] A third potential hazard occurs to human handlers who have close contact with monkeys that have acquired tuberculosis.[19]

III. CAUSES OF LABORATORY INFECTIONS

As we know, the true causes of accidents lie in a concatenation of circumstances rather than the simple, direct effect of one or two external agencies. This is why cause analyses can conveniently follow an epidemiological approach, wherein the total interrelationships and interactions of the host, the accident agencies, and the environment are considered.

In the infectious disease laboratory there are a number of ways in which the major elements present interact with each other. A graphic representation of some of these is shown in Figure 1 where the host (the person) affects and is affected by the environment, physical agents, infectious agents, animals, and even insects in various ways. Of course it is not the interactions themselves that are responsible for accidents; these are normally required for carrying out laboratory functions. But accidents do happen when the sequence is wrong, when the timing is wrong, when the amount is too much or too little, when the wrong choice is made, or indeed, where there is a combination of any of these or other factors.

I have already said that naturally infected monkeys and chimpanzees can transmit diseases to humans in the laboratory. There are, in fact, well over 100 diseases of animals that could conceivably be passed to man. About a dozen of these zoonoses are known to have been transferred from naturally infected laboratory animals to man in the laboratory (lymphocytic choriomeningitis, infectious hepatitis, cat scratch fever, Newcastle disease, psittacosis, monkey B virus, leptospirosis, tuberculosis, malaria, amebiasis, shigellosis, and streptococcal and staphylococcal infections).

Because naturally infected animals can cause laboratory infections we may also expect animals challenged in the laboratory with infectious disease agents to be capable of transmitting infection to humans. Although there are no definitive data showing the frequency with which this happens, we do know that the hazard exists. Moreover, a sizable amount of supportive

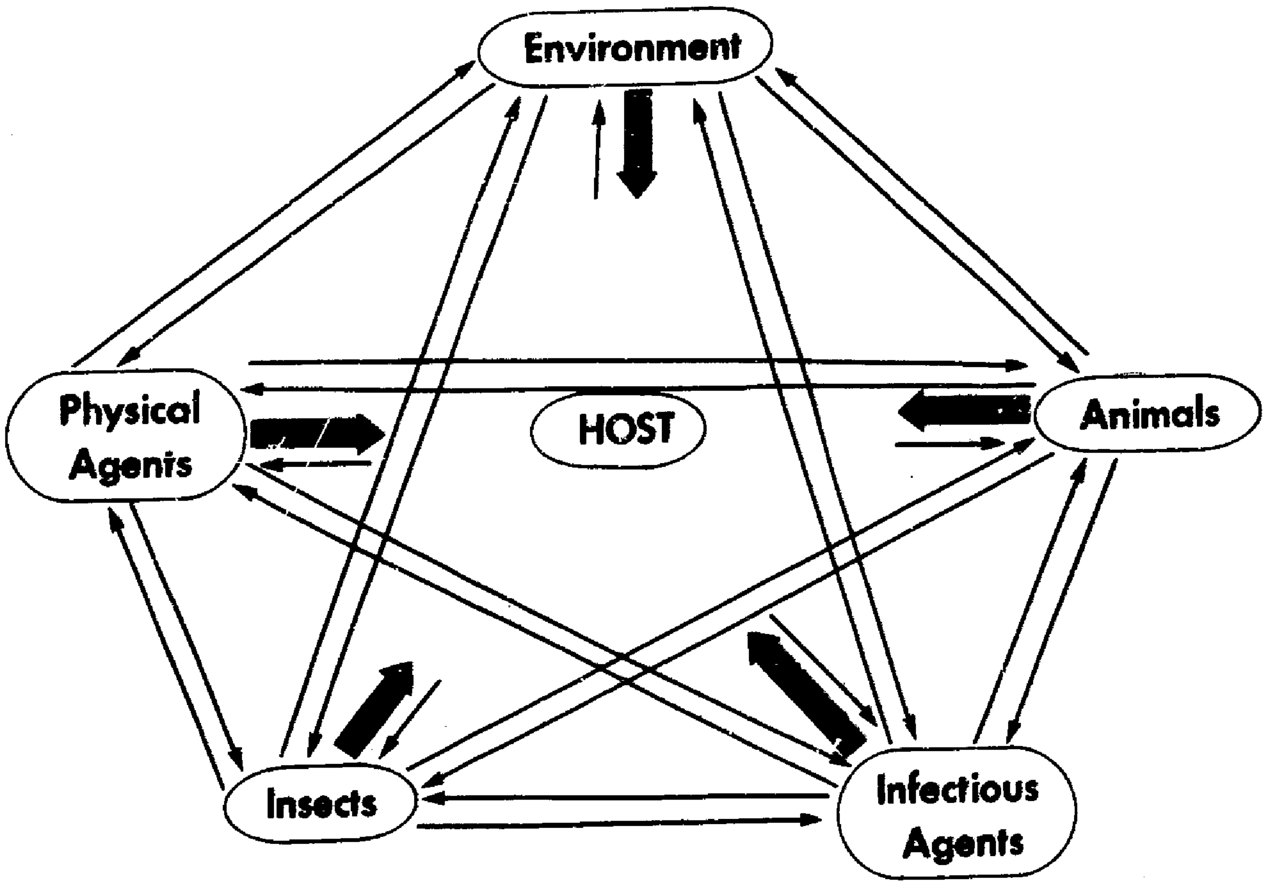

Figure 1. Interrelationships in the Laboratory.

evidence has been provided by animal cross-infection studies.[20] In these studies control animals are usually caged together with infected animals or in adjoint cages. Then periodic tests are made to determine if the controls themselves become infected. The frequency with which cross-infection of this type occurs provides good presumptive evidence of the hazards that may exist for man in that environment.

Infections from animals may be caused by bites or scratches, from contact with contaminated cage debris, or from breathing of air-borne organisms from sick or coughing animals. If animals have been used in aerosol experiments, infectious organisms on their fur may be released to the air to infect animal attendants.

The specific primary causes of laboratory infections fall into two groups. One group, which includes about 20 per cent of the total, consists of recognized accidents that result in the infection of laboratory personnel. The second group, which includes approximately 80 per cent, consists of accidents whose causes are often classified as "unknown" because there were no previously recognized or recorded accidents or incidents that could be shown to have been responsible for the infections. Although this is a somewhat unique situation, that it is true is shown by the fact that the percentage of "unknown" causes has remained reasonably consistent in the various infection surveys (Table IV).

TABLE IV. KNOWN AND UNKNOWN CAUSES OF LABORATORY INFECTIONS

Data Source	Percentage of Accidents	
	Known Cause	Unknown Cause
Paneth, 1915[21]	61	39
Sulkin & Pike, 1951[4]	16-20	84-80
Schafer, 1950[22]	16	84
Survey of 18 countries, 1959[6]	14	86
Fort Detrick, 1955-57	35	65
Fort Detrick mechanical and chemical lost-time injuries	100	0

The five most frequently recognized causes of laboratory infections are: (a) accidental oral aspiration of infectious material through a pipette, (b) accidental inoculation with syringes and needles, (c) animal bites, (d) sprays from syringes, and (e) centrifuge accidents. Together these caused about 12 per cent of some 3700 laboratory infections. A general estimate of the per cent of accidents due to each cause is shown in Table V. Some other commonly recognized causes of laboratory infections are: (a) cuts or scratches from contaminated glassware, (b) cuts from instruments used during animal autopsy, and (c) the spilling or spattering of pathogenic cultures on floors, table tops, etc.

TABLE V. COMMON CAUSES OF LABORATORY INFECTION

Accident Cause	Per Cent of 3700 Infections
Oral aspiration through pipettes	4.7
Accidental syringe inoculation	4.0
Animal bites	1.4
Spray from syringes	1.2
Centrifuge accidents	0.8

Because unsafe acts or unsafe conditions have not been identified in approximately 80 per cent of the recorded laboratory infections, some laboratory procedures and equipment have been suspected of creating hazards. Indeed this suspicion has been confirmed by a number of studies in which the amount of microbial aerosol produced by various laboratory techniques has been measured. Most of the usual techniques have been tested in studies done in this country[16, 23] and in England.[24, 25] They show that most common laboratory techniques carried out in the ordinary manner will produce infectious air-borne particulates. At least one study has shown that these particulates are of a size which will readily penetrate to the human lung if they are breathed.[24] Of course, these results only suggest possible means of laboratory infection. The type of microorganism, its probable infectious dose, its environmental resistance, the resistance of the host, and many other factors would have to be evaluated to accurately define a hazard.

Laboratory infections caused by accidents that can be identified with unsafe acts and conditions or with procedures and techniques that unsuspectingly release infectious aerosols to the laboratory environment illustrate that laboratory safety is a problem of environmental control. The microbe must remain in its environment (test tube, flasks, etc.) and the microbiologist must be externalized from the organism's environment. Although this solution appears simple and straight forward, its application is complex. Microbes capable of causing human infection are not readily detectable in the usual sense; the infecting dose may be odorless, tasteless and invisible to the eye.

Statistical studies of accidents and infections at several large laboratory institutions provide some additional epidemiological data of probable significance in infection prevention. These are summarized as follows:

(a) In general more infections are associated with manipulating cultures than with handling animals.

(b) Laboratory technicians, students, trained professional personnel, and animal handlers, those most closely associated with the infectious operations, are in the greatest danger of becoming infected.

(c) Among those who work directly with pathogens, it is the younger persons with less formal training who are more apt to become infected.

(d) Inhalation of infectious aerosols is by far the most frequent mode of laboratory infection.

(e) The physical form of an infectious agent is related to its hazard level. Dried or lyophilized cultures and infected eggs are more dangerous to handle than liquid cultures or infected blood specimens.

IV. PREVENTION OF LABORATORY INFECTIONS

As with any type of preventive effort, the effort of preventing laboratory-acquired infections should begin by assessing the extent of the problem or by estimating the probable extent of future problems. The potential hazards may result from research being carried on with infectious cultures, from the use of cultures or infected tissues in classroom demonstrations, from clinical diagnostic procedures, or from the use of animals in any laboratory situation. In any case, there must be an understanding and agreement on the dangers by administrators and laboratory directors. Even when the microbiological hazards are understood, the philosophy of scientific

freedom characterized by academic life can often work to oppose the inspections, investigations, and regulatory requirements of a good safety program. Moreover, one is often faced even today with the martyr-to-science complex in which laboratory scientists feel that being infected is part of the job. Particularly in a school situation, legal and moral considerations make this view unacceptable.

Once there is an adequate assessment of the potential microbiological hazards and management is committed to a preventive program, there should be evolved a precise personnel policy regarding occupational health. That is, management should make a series of policy decisions relating to the goals of the safety program and how it is to operate. An adequate list of policy questions have been formulated for those concerned with the construction of laboratory facilities for infectious disease work.[26] Many of these questions apply to the safety policy as a whole. For example:

(a) What level of occupational infection is acceptable to management? Is it desired to attempt to prevent all work-incurred infections, including subclinical infections that can be detected only serologically? Or is management's aim to prevent only those infections that are likely to result in incapacitating illnesses, or only those for which there is no treatment?

(b) To what extent is the control of microbiological hazards to be extended to protect persons on peripheral areas to the laboratory? Public relations, economic and legal considerations are involved here.

(c) What type of supporting medical program is to be provided for persons at risk in the laboratory?

A program for controlling microbiological hazards should begin with a clear concept of the goals, an understanding of the nature of the hazards, and an expression of the policies to be followed in achieving control. By this action, administration establishes responsibility for safety control, includes planning for accident control in all phases of laboratory work, and makes it clear that no job will be considered so important that it cannot be done safely.

In the main, the cardinal tenets of microbiological control will be education, engineering and enforcement. The detailed implementation of safety control can be discussed by considering five important elements. Each element's use is determined by the extent of the microbiological hazards and by management's policy concerning them.

A. MANAGEMENT ASPECTS

Some of the programing and policy responsibilities have already been discussed. Management at various levels must also concern itself with the

proper selection of laboratory employees. This refers not only to technical competence but also to the fact that it may be undesirable to employ persons with certain physical conditions for work with some types of infectious agents. These, of course, are medical decisions.

Management, likewise, should be concerned with providing safety training for laboratory personnel, formulating safety regulations, and establishing methods for adequately reporting and investigating accidents.

The management approach should also attempt to include control of human factors in accident causation and strive to provide an atmosphere wherein personnel may develop attitudes conducive to safe performance. There are no easy answers as to how these aspects are accomplished. Practical experience, however, has shown that they are essential for an accident and infection prevention program to be successful. Of course, good laboratory management includes good safety management. Safety is only one of management's aims, but it is an essential part of any productive enterprise.

B VACCINATION

Vaccination of laboratory personnel is recommended when a satisfactory immunogenic preparation is available. Good immunity is conferred after vaccination against smallpox, tetanus, yellow fever, botulism, and diphtheria. The new living vaccine for tularemia gives excellent protection. Other vaccines such as those for psittacosis, Q fever, Rift Valley fever, and anthrax have been or are being tried experimentally with varying degrees of success.

Vaccines have not yet been developed for a number of human diseases which have been known to occur in laboratory workers. Among these are dysentery, blastomycosis, brucellosis, coccidioidomycosis, glanders, histoplasmosis, infectious hepatitis, leptospirosis, and toxoplasmosis. Moreover, we generally evaluate the efficiency of vaccines for laboratory workers on the basis of their effectiveness in preventing disease in the general population. Two possible pitfalls to this line of thinking should be mentioned. The first is that the laboratory worker may be exposed to infectious microorganisms at a higher dose level than would be expected from normal public exposure. Secondly, this exposure may be by a route different from that normally expected, e.g., respiratory infection with the tularemia or anthrax organism.

C. SAFE TECHNIQUES AND PROCEDURES

Sound fundamental laboratory techniques, well supervised and conscientiously carried out, can do much to achieve environmental control and reduce the hazards of infection. Many procedural rules are obvious because their aim is to prevent direct contact with harmful microbes. Others may be less well understood because their purpose is to prevent air-borne contamination

of the workers' environment at a level where such contamination is not easily or readily detected. Infectious aerosols are like dangerous radiations, except that the former are more difficult to monitor. A list of procedural rules that are widely applicable in infectious disease laboratories follows.[27]

1. Never do direct mouth pipetting of infectious or toxic fluids, use a pipettor.

2. Plug pipettes with cotton.

3. Do not blow infectious material out of pipettes.

4. Do not prepare mixtures of infectious materials by bubbling expiratory air through the liquid with a pipette.

5. Use an alcohol-moistened pledget around the stopper and needle when removing a syringe and needle from a rubber-toppered vaccine bottle.

6. Use only needle-locking hypodermic syringes. Avoid using syringes whenever possible.

7. Expel excess fluid and bubbles from a syringe vertically into a cotton pledget moistened with disinfectant, or into a small bottle of cotton.

8. Before and after injecting an animal, swab the site of injection with a disinfectant.

9. Sterilize discarded pipettes and syringes in the pan where they were first placed after use.

10. Before centrifuging, inspect tubes for cracks. Inspect the inside of the trunnion cup for rough walls caused by erosion or adhering matter. Carefully remove all bits of glass from the rubber cushion. A germicidal solution added between the tube and the trunnion cup not only disinfects the surfaces of both of these, but also provides an excellent cushion against shocks that otherwise might break the tube.

11. Use centrifuge trunnion cups with screw caps or equivalent.

12. Avoid decanting centrifuge tubes; if you must do so, afterwards wipe off the outer rim with a disinfectant. Avoid filling the tube to the point that the rim ever becomes wet with culture.

13. Wrap a lyophilized culture vial with disinfectant-wetted cotton before breaking. Wear gloves.

14. Never leave a discarded tray of infected material unattended.

15. Sterilize all contaminated discarded material.

16. Periodically, clean out deep freeze and dry-ice chests in which cultures are stored to remove broken ampoules or tubes. Use rubber gloves and respiratory protection during this cleaning.

17. Handle diagnostic serum specimens carrying a risk of infectious hepatitis with rubber gloves.

18. Develop the habit of keeping your hands away from your mouth, nose, eyes and face. This may prevent self-inoculation.

19. Avoid smoking, eating, and drinking in the laboratory.

20. Make special precautionary arrangements for respiratory, oral, intranasal, and intratracheal inoculation of infectious material.

21. Give preference to operating room gowns that fasten at the back.

22. Evaluate the extent to which the hands may become contaminated. With some agents and operations, forceps or rubber gloves are advisable.

23. Wear only clean laboratory clothing in the dining room, library, etc.

24. Shake broth cultures in a manner that avoids wetting the plug or cap.

D. LABORATORY SAFETY EQUIPMENT

Both experimental evidence and practical experience have shown that good techniques alone will not prevent infection of laboratory people. Engineering can provide a physical separation of the workers' environment from that of the microorganism. The most important type of safety equipment is the ventilated, protective cabinet.[28] Figure 2 shows one example of a single bacteriological work cabinet that is adequate for many laboratory manipulations. This type of cabinet can be used with attached arm-length gloves, or it can be used with the gloves removed and the air sweeping away from the operator. Figure 3 shows a similar cabinet but without an entrance air-lock and with the glove panel removed. The basic minimum requirements for such a cabinet are: (a) sufficient inward air flow (50 to 100 linear feet per minute) or operation at a negative pressure, (b) filtration of contaminated exhaust air, and (c) means of sterilizing both the exhaust filter and the interior of the cabinet.

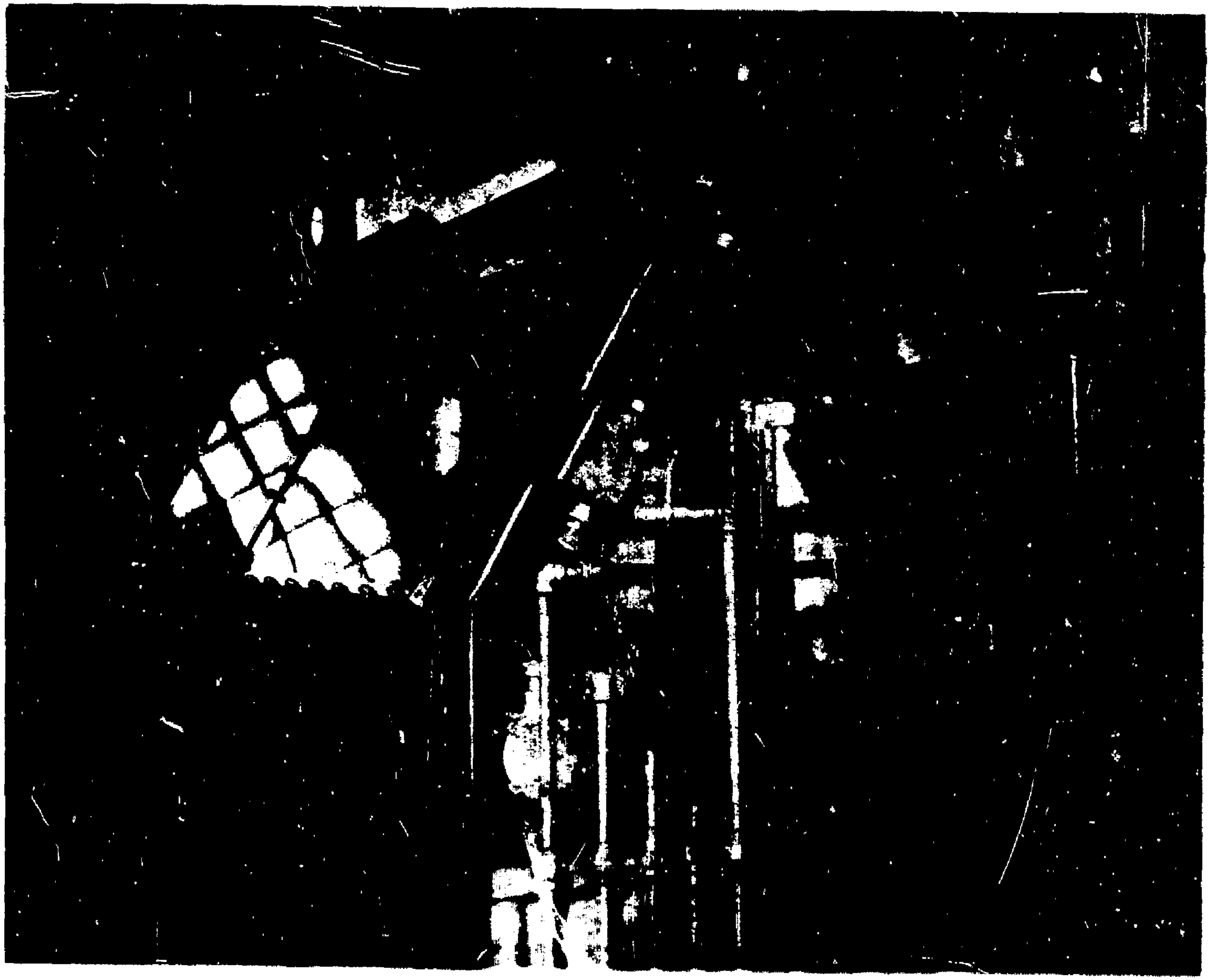

Figure 2. Closed Microbiological Safety Cabinet with Air Lock. (FD Neg C-4172)

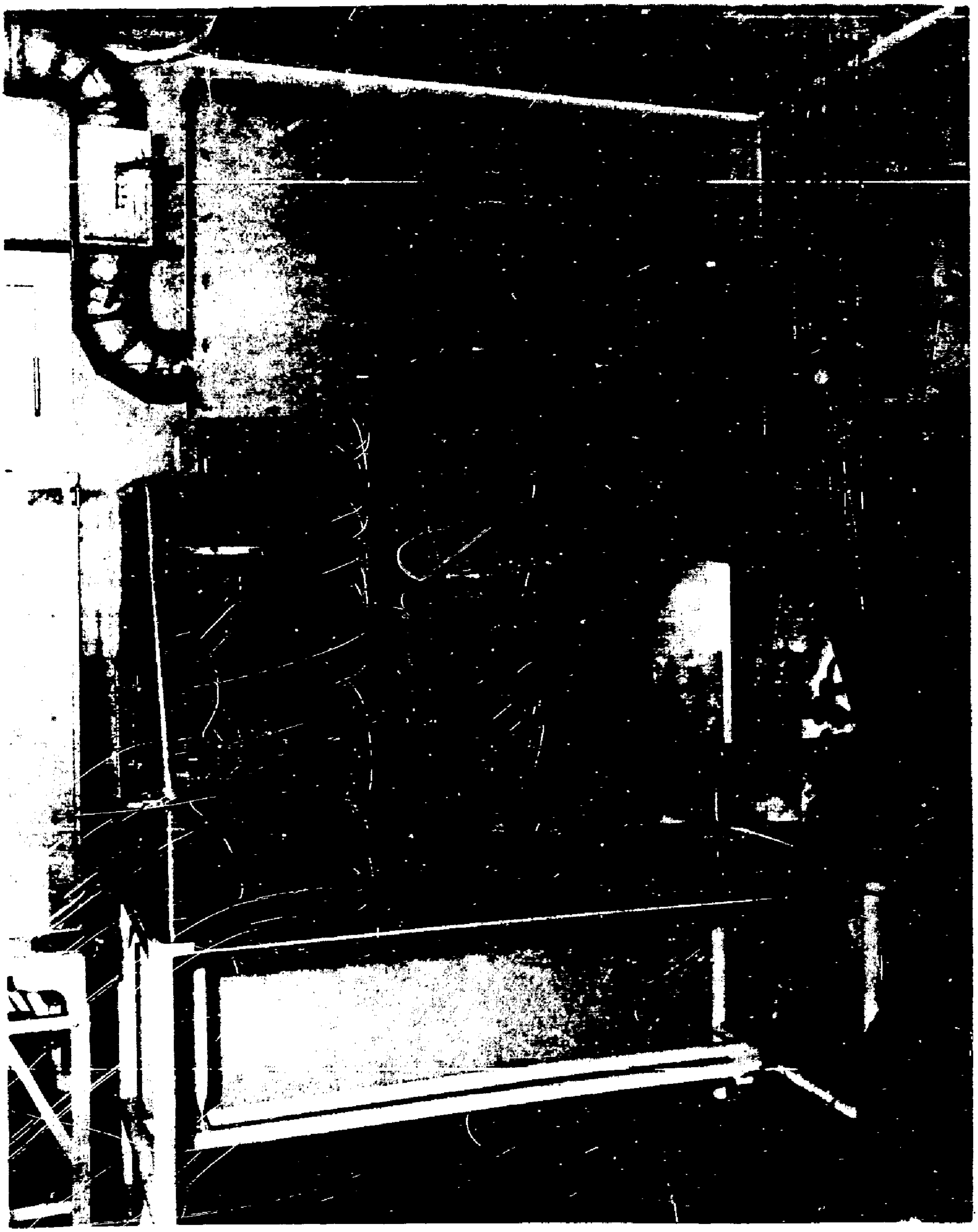

Figure 3. Open Front Microbiological Safety Cabinet. (FD Neg B-6219)

Figure 4 illustrates a more complicated cabinet system for laboratory operations of higher risk. These cabinets are modular units that are gas-tight and can be internally equipped with incubators, refrigerators, deep freezes, and centrifuges. Depending upon their complexity, they can include all equipment needed to carry out microbiological research. These systems, however, are obviously needed only for work at a very high hazard level.

To assist in selecting the proper type of protective cabinets Wedum and Phillips[26] have made estimates of the relative risks of various types of laboratory research and have formulated recommendations for the types of cabinets to use with the agents of a number of diseases. The scale of decreasing laboratory risks starts with a laboratory that wishes to perform any type of experiment with any infectious agent using animals as large as chimpanzees. Use of dry powders of infectious agents presents the next highest degree of risk followed by experiments with highly infectious aerosols. Lower in risk is found laboratory work with highly infectious forms but only in a fluid or liquid culture state.

General realization of the relative order of infectious risks can then be useful in selecting the proper type of cabinet system. Table VI lists a number of disease agents and provides recommendations for appropriate protective cabinets. In general, experiments with microbial aerosols and those using the more serious or more infectious disease organisms are those that should be done in gas-tight cabinet systems. These recommendations are also based on the use of vaccines or toxoids with some diseases.

According to the environmental stability and infectiousness of the pathogens and the method of animal challenge, various degrees of isolation of experimentally infected animals are needed. In a complete containment system animals may be housed within gas-tight cabinets. The next level of containment would be provided by closed ventilated cages such as are shown in Figure 5. A still lower level is provided by the use of open cages screened by ultraviolet radiation.[29]

Other types of containment equipment for specific procedures are available and are recommended for use, especially if gas-tight cabinet systems are not available for centrifuging, blending, shaking, or lyophilizing dangerous pathogens.

Revolving centrifuge tubes may produce microbial aerosols without breaking the tube, if the rim of the tube is wet with the culture during preparation.[25] Although strict attention to technique can avoid this and the use of plastics will reduce tube breakage, these hazards are better avoided by the use of safety centrifuge cups as shown in Figure 6. These, of course, should be loaded and unloaded in a ventilated safety cabinet. Figure 7 shows a ventilated, ultraviolet irradiated chamber for centrifuges used in some Swedish laboratories. Small centrifuges can often be placed in ordinary cabinets for use, but the Sharples super-centrifuge should be enclosed in a specially designed cabinet when used with highly infectious microorganisms. Figure 8 shows a ventilated cabinet designed to accommodate a refrigerated centrifuge.

Figure 4. Gas-Tight Modular Cabinet Systems. (FD Neg C-5360)

TABLE VI. CORRELATION OF ESTIMATION OF RISK WITH RECOMMENDATIONS FOR USE OF PROTECTIVE CABINETS[a]

Disease or Agent	Cabinet System[c] Aerosol Studies	Single Cabinets[d] Aerosol Studies	Single Cabinets[d] Other Techniques
Brucellosis	+++		+++
Coccidioidomycosis	+++		+++
Russian s-s encephalitis	+++		+++
Tuberculosis	+++		+++
Monkey B virus	+++		++
Glanders	++	+++	+++
Melioidosis	++	+++	+++
Rift Valley fever	++	+++	+++
Arboviruses, general		+++	++
Encephalitides, various		+++	++
Psittacosis	++	+++	++
Rocky Mt. spotted fever	++	+++	++
Q fever	++	+++	++
Typhus	++	+++	++
Tularemia	++	+++	++
Tularemia[b]		++	+
Venezuelan encephalitis[b]		+++	+
Anthrax	+++		±
Botulism[b]	++	+++	±
Histoplasmosis		+++	±
Leptospirosis		+++	±
Plague	+++		±
Poliomyelitis	+++		±
Rabies	+++		±
Smallpox[b]	+++		±
Typhoid		+++	0
Adeno, entero viruses		++	±
Diphtheria[b]		++	0
Fungi, various		++	0
Influenza		+	±
Meningococcus		++	0
Pneumococcus		++	0
Streptococcus		++	0
Tetanus[b]		++	0
Vaccinia[b]		++	0
Yellow fever[b]		++	0
Salmonellosis		+	±
Shigellosis		+	±
Infectious hepatitis			±
Newcastle virus		+	0

a. +++ = mandatory; ++ = strongly advised; + = optional, but in absence of a cabinet a few infections will occur; ± = depending upon technique and supervision. 0 = not required.

b. For persons receiving live vaccine or toxoid.

c. Figure 4 or equivalent.

d. Figure 2, 3 or equivalent.

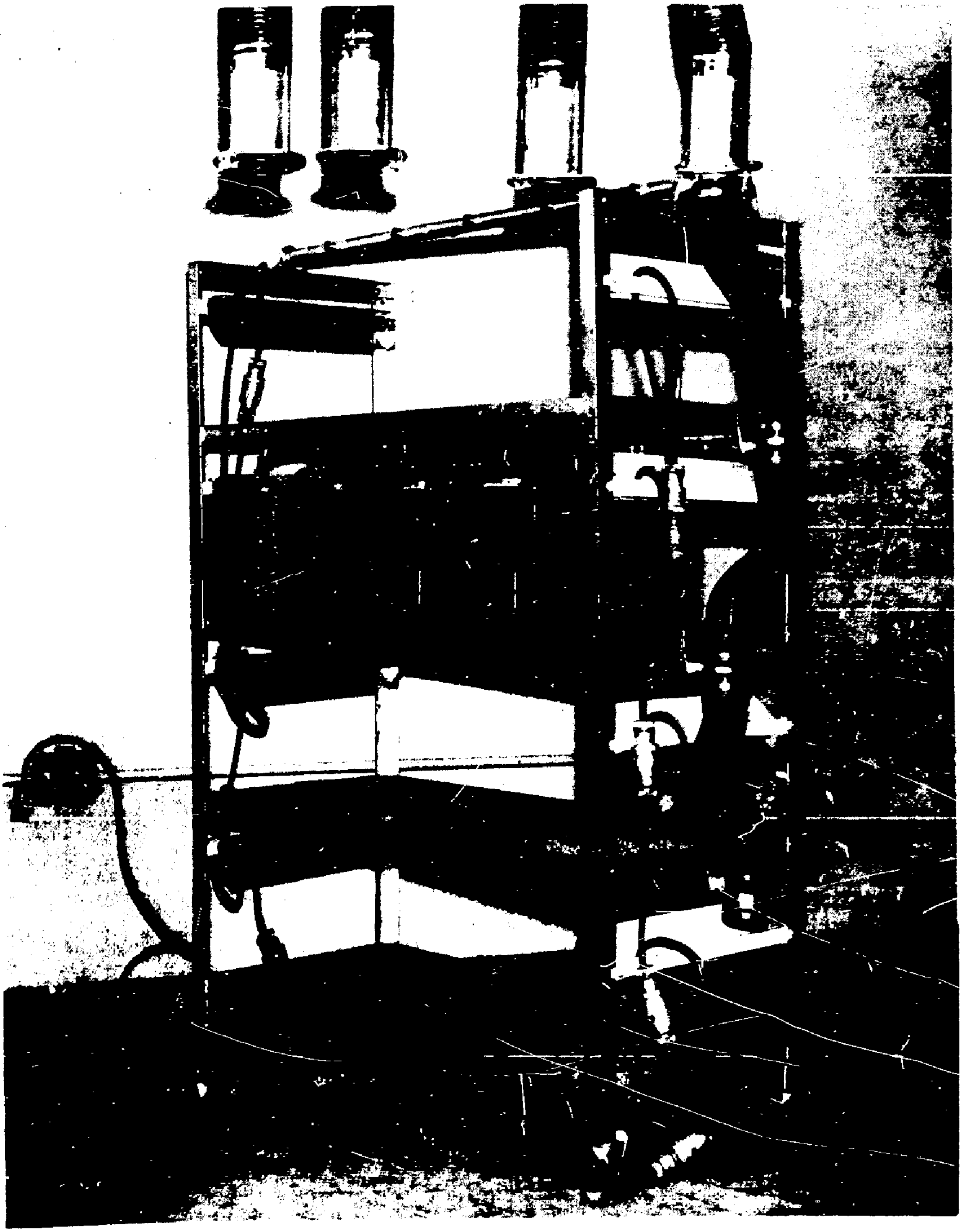

Figure 5. Animal Rack for Ventilated Cages. (FD Neg B-6137)

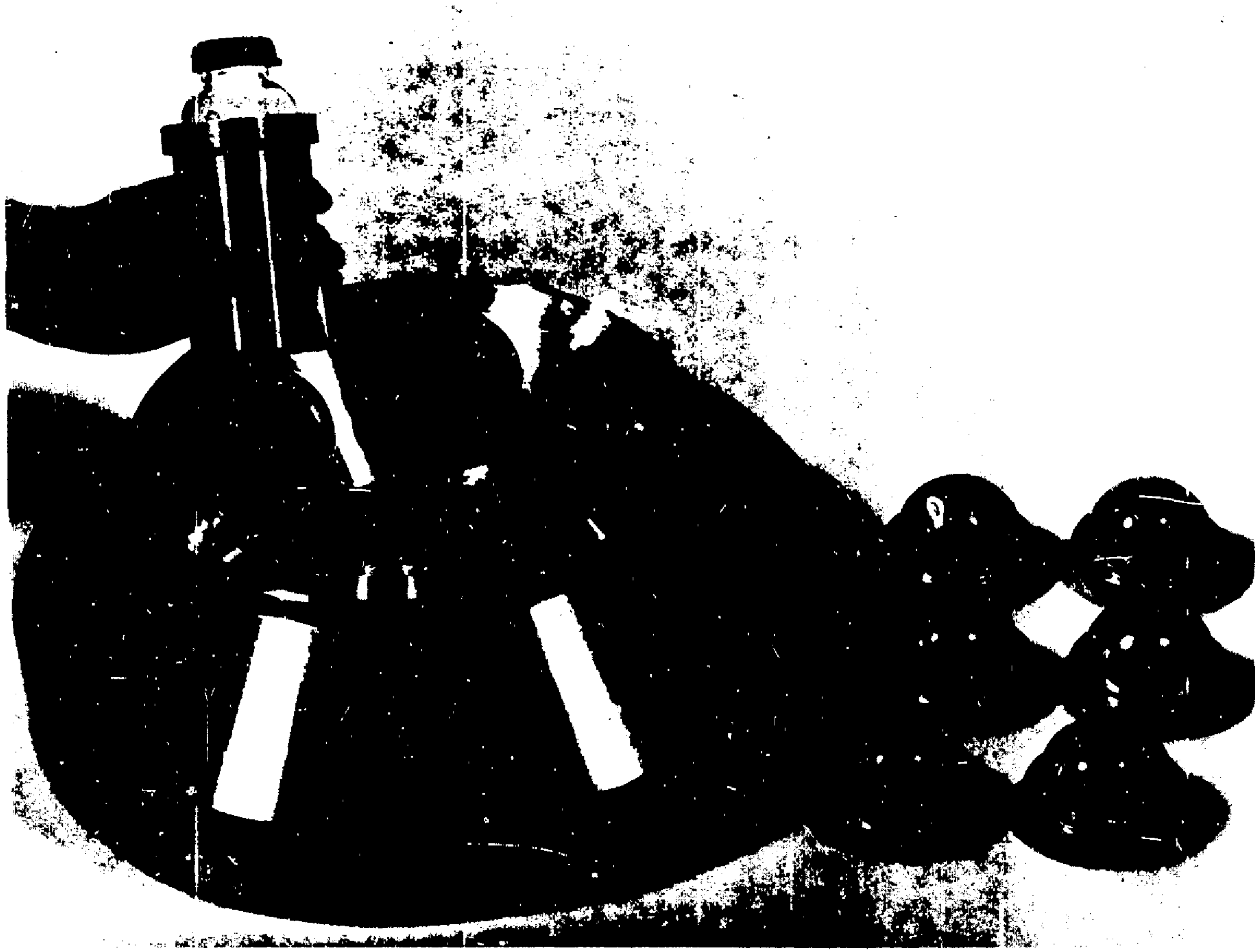

Figure 6. Safety Centrifuge Cup. (FD Neg B-9042)

Figure 7. Centrifuge Cabinet with Pull-Out Drawer. (FD Neg C-7158)

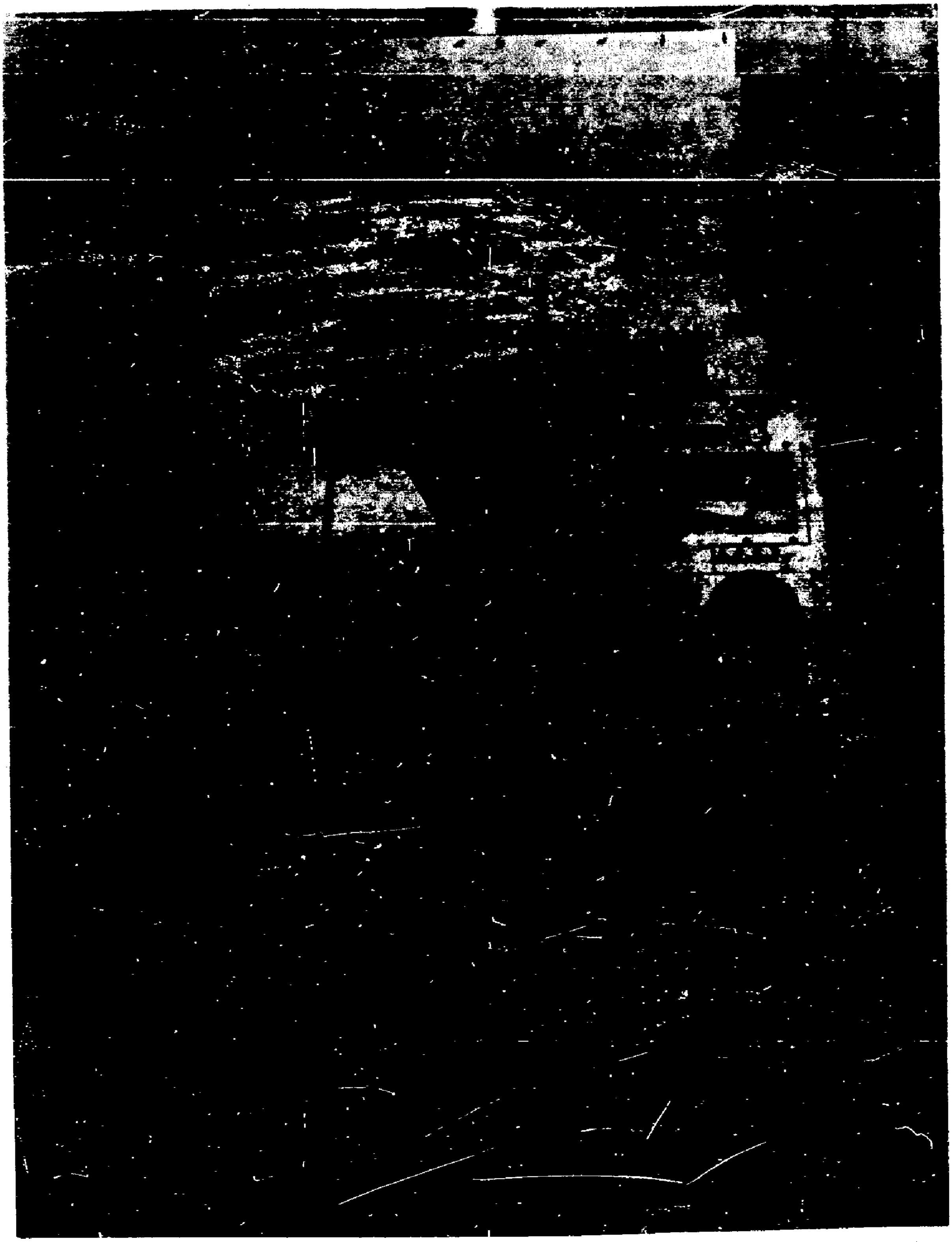

Figure 8. Ventilated Cabinet for Refrigerated Centrifuge. (FD Neg B-9317)

The shaking of cultures during incubation can present substantial infectious risks if flasks or tubes break or if the stoppers fall off. If shakers are not enclosed in ventilated cabinets, aerosol tight shaking containers with viewing windows should be constructed for each shaker.[30]

A lyophilizing apparatus is likewise hazardous when used with pathogens. It should either be operated in a closed cabinet or constructed so as to be sterilized without dismantling. In either case a bacterial filter should be located in the exhaust air line before the vacuum pump.[31]

E. BUILDING DESIGN

Modern construction criteria applied in building laboratories and animal rooms can do much to reduce infectious risks and to prevent laboratory infections. Some of these features suggested for inclusion in new or renovated facilities have been discussed along with typical recommended floor plans.[32]

Some engineering features commonly used for microbiological environmental control in infectious laboratories and animal rooms include (a) ventilated cabinets and cages to contain microbes at their point of use; (b) differential, increasingly negative air pressures as one moves from clean areas to those of greater infectious risk; (c) appropriately effective filtration of air from rooms, cabinets, and ventilated cages; (d) change rooms and showers for personnel; (e) ultraviolet air locks and door barriers to separate areas of unequal risk; (f) treatment of contaminated liquid effluents; (g) room arrangement or layout to achieve traffic control along a clean-contaminated axis; and (h) an effective intercommunication system. For those faced with initiating a design plan, the problem is one of determing which of the above items are to be used and to what extent.

Moreover, it is usually necessary to make these determinations in the early stages of planning a new or renovated laboratory facility. The problem is difficult. The dangers are that the facility design will provide more protection than needed, less protection than needed, will fail to protect the surrounding community, or will be too inflexible in the future. Consideration of some basic policy decisions before the design is begun will provide a basis for selecting specific construction details. A comprehensive list of such policy questions has been published.[28]

Control of air in the laboratory is especially important because the most common source of occupationally-acquired infection is the inhalation of accidentally or experimentally-produced microbial aerosol. Control should begin where the aerosol is formed; at the laboratory bench level. This is why there should be emphasis on primary containment devices such as ventilated cabinets. To the extent that the microbiological hazards are controlled at their source, other building features such as differential air pressures in rooms, protective respiratory equipment, ultraviolet irradiation, and personnel showers become less important in protecting laboratory employees.

This consideration also illustrates the necessity for a reasonable balance in laboratory equipment and facilities for safety. An example of inconsistent or imbalanced design would be the biological filtration or incineration of air from a room in which there are no ventilated microbiological safety cabinets or animal isolation cages. If the room air is at a hazard level requiring its filtration before discharge, it is inconsistent to have unprotected human occupants in the room.

Major decisions, then, in selecting engineering safety features should be based on the fact that safety begins at the laboratory work surface because that is where most infections originate.

V. EDUCATION IN MICROBIOLOGICAL SAFETY

In spite of all that has been written and said about the prevention of accidents, it remains undisputed (as well as unsolved) that the single most important need is that of convincing people that safety is a way of life, that it holds immediate and long range personal benefits, and that it is an essential part of a well developed, orderly, full and enjoyable life. As Brody[33] has stated, "The psychology of safe behavior is no more and no less than the psychology of human behavior in general." Grimaldi[34] moreover, has stated, "Although safety programs are for the people, they are not of the people or by the people--unfortunately." These statements hold significant meanings for laboratory safety by emphasizing that, in spite of the considerations, approaches, and equipment discussed thus far; human factors in accident prevention occupy a dominant position and the educational process is an essential brick in the cornerstone of safety.

Moreover, as we are speaking primarily about safety in the campus situation, education takes on a double significance. Ultimately, the responsibility for the safety of the individual working in the infectious disease laboratory rests in some way with the teaching institution that provided his initial training in laboratory procedures. Endowing the student with heuristic desires and technical knowledge is not enough. He must be taught how to use the instruments and apparatus of the laboratory. He must, in the learning process, be made to understand the importance of the manipulations and impressed with the notion that a good scientist is also a safe scientist. Too often school authorities have solved infectious hazards problems and therefore avoided the need for microbiological safety education simply by forbidding the use of pathogenic agents in the school's laboratories.

This, of course, is "begging the question." It is true that not all microbiologists handle or need to handle infectious organisms in their work. But telling the student who is taking a course in infectious diseases that

if, later in his career, he is required to handle pathogens, his employer or someone else will give him the proper instructions is merely academic "buck-passing." For those who will agree that microbiologists and others who work with infectious microorganisms should be given every opportunity to protect themselves from acquiring occupational diseases, these questions should be asked: Should not safety education in the hazards associated with handling highly virulent microbes be included in the college curriculum? The modern educational system is expected to produce professional people who have the knowledge and the skills which will enable them to be continuously effective in their chosen fields. If safe behavior is indeed a concept of life and if ~~important~~ contributions to ones later attitudinal outlook are formed early in life, is it realistic to wait until after the completion of professional training to institute education in safety?

VI. CONCLUSIONS AND FUTURE PROSPECTS

As we have seen, the use of agents and animals often leads to occupational disease among laboratory people. From the point of view of each safety administrator, it is most important that an evaluation be made of actual, potential, or future microbiological hazards before deciding if and how much of a prevention effort is required.

It has been principally during the last two decades that attention has been given to the problem of correcting or reducing laboratory-acquired illnesses. Former traditions of personal sacrifice are gradually becoming outdated by economic, moral, and legal pressures. Also, in the last few years, it has become eminently clear that laboratory determinations will be accurate only if controlled to the extent that concurrent culture cross-contamination or animal cross-infection can be prevented. This has prompted research helpful in developing techniques and methods which reduce human infectious risks in the laboratory. The most important single conclusion from this research is that preventing the release of accidental microbial aerosols at the laboratory working surface through careful techniques and through the use of containment devices is the best way of achieving microbiological environmental control.

The specific tools for controlling microbiological hazards are:

(a) The required management supports and administrative techniques of reporting, analyzing, selecting, regulating, and training.

(b) The use of correct techniques.

(c) The use of safety equipment.

(d) Properly designed laboratories.

(e) Vaccination of personnel.

In a campus situation it is essential that the school discharge its responsibility for the safety education of students who are exposed to infectious disease hazards. Increased national expenditures for education and increased emphasis on microbiological research portends an increasingly greater demand for microbiological safety programs in laboratories in order to protect potentially exposed students, researchers, and scientists.

Education in laboratory safety methodology requires, as a background, an adequate body of facts about laboratory hazards, their prevention, and most particularly, their causes. In a time when there is a clearly recognized shortage of educators and teachers it is appropriate that scientific methodology be applied in efforts to control and reduce laboratory infections among teachers, researchers, and students. Moreover, future demands on the educational system signal a need for research information on this subject for use by educators.

Of significance, for example, is the trend toward team research which persons trained in fields other than microbiology use infectious cultures as tools in the solving of life-science problems. Should the effort to isolate and identify virus strains as the etiological agents of certain cancers be successful to any degree, the need to protect research workers handling such strains would come under consideration. Perhaps it should be considered now. Likewise, in the space satellite research program it has been recognized that uncontrolled transfer of microbes between planets is undesirable. In the medical field, a more immediate hospital problem is that of the spread of staphylococci and other infections among hospital personnel and patients. The principles of environmental control applicable in laboratory microbiological safety are helpful in solving these problems.

Before microbiological safety control can be successfully integrated into needed areas in colleges and universities, it will be necessary, through safety education, to impart knowledge about these hazards to the university staff and through them to the students.

The task promises to be formidable but nonetheless it should be made a part of future planning. Because of present emphasis on the teaching of science, many new and enlarged laboratories and teaching facilities are being constructed. Expanded courses in specialized areas of microbiology are being planned and larger teaching staffs are being sought. Now is certainly the time to incorporate microbiological laboratory hazard control programs into needed areas and to institute the educational process to this end.

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